ON THE EXCRETION AND THE METABOLISM OF HISTAMINE

BY

KERSTIN LINDAHL-KIESSLING

UPPSALA 1960
ALMQVIST & WIKSELLS BOKTRYCKERI AB

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The present publication is based on the following papers:

I. Tubular excretion of histamine in the hen. (Together with I. Sperber.) Acta Physiol. Scand. 1956, *36*, 13.

II. Some characteristics of the renal tubular transport mechanism for histamine in the hen. (Together with I. Sperber.) Acta Physiol. Scand. 1958, *42*, 166.

III. Methylation of histamine by mouse liver *in vitro*. Acta Physiol. Scand. 1958, *43*, 254.

IV. On the biological methylation of histamine. Arkiv Kemi 1958, *13*, 149.

V. On the biological methylation of histamine. Acta Chem. Scand. 1958, *12*, 1690.

VI. On the biological methylation of histamine. Acta Chem. Scand. 1958, *12*, 2050.

VII. The histamine methylating enzyme system in liver. Acta Physiol. Scand. 1960, *49*.

VIII. *In vitro* studies on histamine metabolism in placenta. Arkiv Kemi 1960, *16*, 1.

IX. Paper-chromatographic indication of imidazole-4(5)-acetic acid and 1-methyl-imidazole-4-acetic acid as histamine metabolites in human placental tissue. Arkiv Kemi 1960, *16*, 15.

In the text these papers will be referred to by the above Roman figures.

The manifold and drastic pharmacological actions of histamine have created a great interest in this substance ever since its discovery some fifty years ago. Its occurrence in the tissues and body fluids and its release in physiological and pathological conditions like allergy and anaphylactic shock have been exhaustively described. Yet the role played by it in normal physiology or under special conditions characterized by histamine liberation is still obscure. The stimulation of gastric secretion constitutes perhaps an exception. These problems have recently been treated in review articles (Tabor 1954, Rocha e Silva 1955, Ciba Foundation Symposium on Histamine 1956, Paton 1958, Riley 1959, Kahlson 1960) and the above remarks serve merely as a reminder of the importance ascribed to histamine.

As a natural consequence the urinary excretion and the enzymatic inactivation of histamine in the body have been intensely studied in many laboratories. The work reported here represents an effort to throw additional light on these processes.

As regards the excretion, quantitative changes of the urinary histamine have been repeatedly demonstrated under various conditions but to my knowledge the mechanism of excretion has not been studied. It is obvious that histamine may be filtered in the glomeruli in so far it exists in a freely diffusible form in the plasma, which is, however, a matter of controversy. The possibility of its secretion and/or reabsorption in the tubules have also to be taken into consideration, especially as the tubular secretion may be considered to represent a defence mechanism of the body for quickly getting rid of toxic substances.

The principal trends of the enzymatic mechanisms for inactivation of histamine are rather well outlined. Thus three different enzymatic processes are known which seem to account for the main part of the histamine catabolism in a great number of species, namely (1) oxidative deamination to imidazoleacetic acid,[1] the greater part of which is conjugated with ribose to imidazoleacetic acid riboside, (2) methylation to methylhistamine followed by oxidative deamination to methylimidazoleacetic acid and (3) acetylation to acetylhistamine. The relative importance of the different enzyme systems in man may be illustrated by some data from Schayer and Cooper (1956). After injection of 1 μg C^{14} histamine per kg b.w., analysis of the urine for metabolic products of histamine gave the following values, expressed as per cent of total recovered radioactivity: unchanged histamine 3 %, imidazoleacetic acid 10 %, imidazoleacetic acid riboside 19 %, methylhistamine 6 %, methylimidazoleacetic acid 45 %, and acetylhistamine < 1 %. All values are averages of three experiments.

Diamine oxidase, which catalyses the formation of imidazoleacetic acid, and the acetylating enzyme have been partly purified and described. However, the individual enzymes responsible for the formation of methylhistamine and the oxidative deamination of this substance to methylimidazoleacetic acid are mainly unknown.

From considerations of convenience, the following discussion of the above problems will be divided into three sections. In the first section the excretion of histamine will be treated. In section 2 the principal subject is the methylating enzyme system. In section 3 the interest is centred around the histamine metabolism in human pregnancy.

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[1] The complete names for these compounds are, respectively, imidazole-4(5)-acetic acid, 1-β-D-ribofuranosylimidazole-4(5)-acetic acid, 1-methyl-4-(β-amino-ethyl)imidazole, 1-methylimidazole-4-acetic acid, and 4-(β-acetylaminoethyl)imidazole.

1. Removal of histamine by renal excretion

The continuous appearance of free histamine in the urine in several species has been unequivocally demonstrated (for references see Rocha e Silva 1955). The first and perhaps the only report giving quantitative data concerning the relationship between the amount of histamine in plasma and the amount excreted in the urine were given by Adam and his co-workers. They found that marked increases in free histamine excretion in the urine follow slow intravenous infusions of histamine, but that only about one per cent of the injected histamine appears in the urine independent of the rate of infusion (Adam *et al.* 1954). They suggested that the urinary histamine is derived from the plasma and concluded that the release of histamine from the tissues and its absorption from the gut may conveniently be studied by determination of the urinary output. Further Roberts and Adam (1950) determined the normal output in man at about 20 μg per 24 hrs and in another investigation (Adam *et al.* 1957) the venous plasma concentration of histamine was found to be less than 1×10^{-3} μg/ml.

The studies by Adam and his colleagues (1954) indicate that the removal of plasma histamine is very efficient. No histamine was detectable in the venous plasma with the methods used, even during continuous infusion of histamine at a rate high enough to evoke pronounced gastric secretion and other biological effects. Further, the comparatively low concentration of histamine in the urine during intravenous infusion demonstrated in the experiments above may imply that histamine is transported bound to some blood component, or is reabsorbed in the tubules.

More recently Schayer demonstrated that in the rat urinary histamine may originate not only from the plasma but from histidine, decarboxylated in the kidney itself (Schayer *et al.* 1954). These findings have later been extended to dogs. Radioactive histamine, imidazoleacetic acid and methylimidazoleacetic acid were found to be excreted after injection of C^{14} histidine into the renal artery (Lindell and Schayer 1958*c*). The authors suggested that the newly formed histamine is excreted immediately without having entered the general circulation. Then this histamine must have been excreted into the urine by the tubular cells.

The problem of the mode of excretion of histamine by the kidney has been studied in papers I and II. In these papers an attempt was made to see whether histamine could possibly be excreted into the urine by tubular excretion. In the chicken the experimental analysis of this problem can be undertaken without the complications involved in the use of the ordinary clearance technique, because of the fact that birds have a renal portal circulation (Sperber 1946, 1948*a*).

Any substance infused into a leg vein of the chicken passes at least partly through the renal peritubular capillary network before entering the general circulation. As a compound which is excreted by the tubules will be partly transferred to the tubular urine during this passage, the excess excretion on the side of the infusion is then a measure of the efficiency of the tubular excretion. A method that makes use of this circumstance has been worked out and the principles of the experimental set-up have been discussed by Sperber (1948*b*). He concluded that an excess on the injected side higher than 10 per cent of the injected dose indicates tubular excretion.

In the experiments described in paper I, 10 to 650 μg histamine were injected into

a leg vein of the hen and the urine was collected separately from the ureters and analyzed for histamine on atropinised guinea pig gut in the conventional manner. It will be noted that the method used does not differentiate between histamine and N-methylhistamine.

In each case a considerable part of the histamine was rapidly excreted on the side of the injection, and the amounts excreted on the other side were very small. The simultaneous inulin excretion was fairly equally divided between the two kidneys. The pH of the urine appeared to have no influence upon the histamine distribution (Paper II). The rate of uptake of histamine by the tubular cells was high: an injection rate of 667 μg per min was not sufficient to diminish the percentage excess on the injected side.

The histamine excess on the injected side was in every case about as large as the simultaneous phenol red excess. As phenol red is well known to be efficiently excreted by the tubules, the evidence for tubular excretion of histamine is very strong.

Several functionally discrete mechanisms for renal tubular secretion have been described. Thus all organic acids known to be actively secreted by the renal tubules are usually considered to be transported by a common mechanism. They generally depress the excretion of one another, competing for secretion by the renal tubular cells (for a review see Sperber 1959). The second mechanism is concerned with the transport of a number of amines and organic bases. Tubular excretion of e.g. N-methylnicotinamide, thiamine, piperidine, guanidine, methyl guanidine, choline, priscoline and darstine has been demonstrated in the mammalian and avian kidney. Several of these cations have been shown to depress the excretion of one another, when occurring in sufficient amounts in the blood. The basic dye cyanine #863 is a potent inhibitor of the tubular transport mechanism for these bases in the dog and the chicken (for a review see Peters 1960).

The experiments presented in paper II give evidence that the tubular excretion of histamine takes place through the transport mechanism which is utilized by other organic bases, excreted by the avian and mammalian tubules. Thus the histamine excretion is depressed by priscoline and, although less markedly, by cyanine #863. However, Rennick *et al.* (1956) found that in the chicken the cyanine inhibition of tetraethylammonium excretion was more delayed and less striking than in the dog, which is in accordance with the results concerning histamine. The tubular excretion of histamine was apparently uninfluenced by inhibition of the above-mentioned transport system for phenol red and allied substances.

Even fairly closely related animals may show considerable differences with regard to the tubular excretion of a few substances, but on the whole the mechanisms for tubular excretion seem to be similar among all vertebrates examined. In view of these considerations it may be of some interest to examine current views of the excretion of histamine, and also methylhistamine, in mammals.

In a recent investigation on the renal removal of histamine in the dog, Lindell and Schayer (1958*a*, *b*) found that although histamine in the blood was extracted very efficiently by the kidney (70–80 %), only a moderate amount appeared in the urine (14–18 % of the extracted amount). Injection of aminoguanidine was followed by a diminished extraction ratio and a somewhat increased urinary output. The results were interpreted as indicating that injected histamine was taken up by the tubular cells and metabolized but was not, or only to a small extent, secreted by these cells, the free histamine in the urine presumably resulting from glomerular filtration. This investigation shows that injected histamine is rapidly metabolized in the dog and

that this is undoubtedly the main way for removal of at least small doses. The existence of a tubular secretory mechanism for histamine also in the dog can, however, not be excluded, which might function when big doses are injected (cf. Alexander 1944).

It would be very interesting to know whether the transport mechanism handling several organic bases in the dog (cf. above) influences the renal mechanism of histamine removal similar to what is undoubtedly the case in the chicken. The data obtained by Lindell and Schayer indicate that there exists an efficient mechanism for the rapid uptake of histamine into the tubular cells. Further if histamine formed in the kidney is excreted without having entered the general circulation (Schayer *et al.* 1954, Lindell and Schayer 1958*c*, quoted above) this seems to imply that there must exist also an output mechanism. Anyhow their data concerning methylhistamine suggest very strongly that this substance is excreted by the tubular cells.

During the last few years several authors have been concerned with the urinary excretion of histamine in man (Dunér and Pernow 1956, 1958, Mitchell 1959). Dunér and Pernow suggested that histamine is excreted by filtration from the plasma, as they could establish a correlation between the histamine concentration in blood and the excreted amount. However, it is not known to what extent the histamine concentration in blood may be equalled by filtrable plasma histamine. Apart from this question, a correlation between plasma histamine and urinary histamine is also compatible with the idea of tubular excretion of histamine at small tubular loads.

Thus it must be concluded that the mechanism of histamine excretion in mammals is still to same extent obscure. However, several investigations show that histamine is taken up very efficiently by the mammalian kidney and thus the existence of a mechanism is established, which may be regarded as the first step in a secretion mechanism.

2. Enzymatic removal of histamine

The quantitatively most important way to render histamine biologically inactive is the enzymatic degradation. The development in this field has been reviewed by Zeller (1951) and Tabor (1954). More recent investigations in the living animal have been reviewed by Schayer (1959). Therefore only a few relevant points will be discussed here.

Several different routes for the histamine catabolism have been discussed, but three of them are so far considered to be quantitatively most important: (1) oxidative deamination to imidazoleacetaldehyde which is further oxidized to imidazoleacetic acid and occasionly conjugated with ribose, (2) acetylation to acetylhistamine and (3) methylation to methylhistamine which is oxidized and deaminated to methylimidazoleacetic acid.

The interpretations of the first-mentioned reaction are somewhat confused at present. It appears that there are several different enzyme systems that may catalyse the oxidative removal of the side-chain amino group of histamine. The best known of these is the classical histaminase (Best and McHenry 1930) or diamine oxidase, usually prepared from pig kidney, which oxidizes mainly diamines and is strongly inhibited by aminoguanidine. Two plasma oxidases, tentatively called spermine oxidase (see Tabor *et al.* 1954) and benzylamine oxidase (see Blaschko *et al.* 1959) are also inhibited by aminoguanidine. They accept monoamines and, although less readily, histamine as substrates. An enzyme which oxidizes monoamines as well as diamines and histamine and is inhibited by aminoguanidine has been found in pea seedlings (Kenten and Mann 1952, Mann 1955). In contrast the classical intracellular monoamine oxidase (Hare 1928, Blaschko 1952), most commonly prepared from liver, is not inhibited by aminoguanidine and it does not, or very slowly (Zeller, Stern and Blanksma 1956) act on histamine. Finally an enzyme from mouse liver which attacks diamines and histamine but is inhibited only by iproniazid and not by aminoguanidine has been described (Kobayashi 1957). For detailed information the reader is referred to the recent review by Blaschko *et al.* (1959).

Acetylated histamine is found in the urine in many species, especially after ingestion of meat or protein rich food (Rocha e Silva 1955). The acetylation may be considered as a "protective synthesis" (see Harper 1959). It is catalysed by an enzyme that mediates the transfer of acetyl from acetyl coenzyme A to various aromatic amines and histamine (Tabor *et al.* 1953). The enzyme seems to contain essential sulfhydryl groups.

Methylation is another common "protective synthesis" or detoxication process for cyclic compounds. As early as 1926 Novello, Harrow and Sherwin investigated the possibility that the imidazole ring should be methylated *in vivo*. However, the methylating and oxidative metabolic pathway of histamine was not discovered until some thirty years later. It was first demonstrated *in vivo* by Schayer and his coworkers, who could establish that part of injected histamine was methylated at the imidazole nitrogen remote from the side chain and thereafter oxidized and deaminated to methylimidazoleacetic acid, which appears in the urine (for a review see Schayer 1959). The enzyme system was demonstrated *in vitro* by Kobayashi and

Schayer (1956), who isolated radioactive methylimidazoleacetic acid from an incubate of cat-liver homogenate with C^{14} histamine.

The formation of the postulated intermediate, methylhistamine, was first demonstrated *in vitro* in mouse-liver slices by chromatographic methods (III). The nature of the enzyme which in the body catalyses the oxidation of methylhistamine to methylimidazoleacetic acid is not known. However, the process seems to be unaffected by aminoguanidine (see Schayer 1959). Thus the mouse-liver enzyme or possibly the benzylamine oxidase referred to above may be considered here. These amine oxidases have been shown to act upon methylhistamine *in vitro* (Kobayashi 1958, Blaschko 1959).

The primary aim of the experiments presented in paper III was the identification of methylimidazoleacetic acid and methylhistamine. Hence methods were chosen which can be regarded as only semi-quantitative. However, as the results were very distinct, some conclusions concerning quantitative relationships could be drawn. It was found that aminoguanidine was not an inhibitor of the methylating enzyme system. Isonicotinyl-isobutylhydrazine, a monoamine oxidase inhibitor, blocked the formation of methylimidazoleacetic acid but did not completely prevent the methylation process. These properties of the enzyme system could be expected on account of the findings *in vivo* by Schayer and his co-workers.

Of special interest was the observation that aerobic conditions seemed to be necessary for the methylation process. This was interpreted as an indication that the methylation was dependent on a continous supply of ATP.

Further studies were possible when an enzyme preparation was made available which initiated the methylation of histamine without subsequent oxidation (paper IV). The methylating enzyme was obtained from mouse liver by removal of inert protein by high-speed centrifugation and heat precipitation at pH 5.6. It is not known whether the oxidative enzyme system was inactivated or actually removed by the fractionation procedures.

The experiments reported in paper V demonstrate that methionine, labelled on the methyl group, and histamine in the presence of ATP, give rise to radioactive methylhistamine when incubated with the crude enzyme preparation obtained from mouse liver. In these experiments the isotope dilution method for quantitative determination of histamine and its metabolites, developed by Schayer and his co-workers (see VII) was used. However, addition of methionine alone or other methyl donors, known to take part in the transfer of methyl groups, such as betain (c.f. Ericson *et al.* 1955) and methionine sulfoxide (Valyi-Nagy 1947) were not sufficient to bring about a histamine methylation (VII).

At this time S-adenosylmethionine was made accessible by a preparative method, elaborated by Schlenk and De Palma (1957). The occurrence of this substance had been proved by Cantoni some time earlier and its function as a transmitter of methyl groups from methionine to nicotinamide and guanidinoacetic acid was established (for a review see Cantoni 1957).

The experiments described in paper VI show that S-adenosylmethionine was indispensable for the histamine methylation catalysed by the mouse-liver enzyme. In comparison with the above-mentioned data it was obvious that S-adenosylmethionine functions as the direct methyl donor.

This finding made it possible to define the conditions necessary for *in vitro* studies of the methylating enzyme and thereby the unknown route of histamine metabolism.

Introductory studies on the histamine methylating enzyme, hereafter called hista-

mine methyl-transferase are described in paper VII. (This paper also contains a brief review and on some points an extension of the experiments in papers IV to VI.)

It is quite clear that a comparative analysis concerning the activity of an enzyme cannot be undertaken unless the initial reaction velocity in each case can be at least approximated. Therefore suitable conditions for the methylating reaction had to be worked out. Standard conditions were finally adopted which permitted the reaction velocity to be considered practically linear for 30 minutes. Studies concerning the substrate and inhibitor specificity of the histamine methyl-transferase, briefly summarized below, were then undertaken (paper VII).

It is now known that many substances are methylated by the mediation of S-adenosylmethionine, but so far no other substrate than histamine has been found for the methyl-transferase described here.

The effect of some SH inhibitors on the methyl-transferase was investigated. Chloroacetophenone, iodoacetamide, N-ethylmaleimide, phenylmercuriacetate and *p*-chloromercuribenzoate (but not *o*-iodobenzoate) inhibited the enzyme activity. The inhibition by the mercaptide forming reagents was reversed by addition of glutathione but not the inhibition caused by alkylating reagents. In all cases the inhibition could be prevented by glutathione. The activity of some enzyme batches was stimulated by glutathione, while other preparations had maximal activity without addition of sulfhydryl compounds. It was suggested that the enzyme has essential SH groups.

The amine-oxidase inhibitors, aminoguanidine and iproniazid, had no influence on the histamine methyl-transferase.

The localization of the histamine methyl-transferase within the cell was also investigated (VII). The mitochondrial and the nuclear fractions seem to be devoid of enzyme activity, since this can be quantitatively recovered from the supernatant fraction after centrifugation of a homogenate at 100,000 × g. However, the possibility of leakage from subcellular particles, e.g. the microsomes, cannot be entirely excluded.

Histamine methyl-transferase was demonstrated in the liver of human beings, hamster, rabbit, mouse, pig and cat but not in the liver of the rat (paper VII). This is in agreement with the findings by Schayer who found that man, the dog, the rabbit, the guinea pig, the mouse and the cat excrete methylated histamine derivatives but not the rat (see Schayer 1959). Later Westling (1958) has found significant quantities of methylhistamine in rat urine. He reports a sex difference in the histamine metabolism of the rat. Provided that histamine methyl-transferase occurs also in the rat, there must be other points, e.g. with regard to the localization or the requirements for enzymic activity, which differ from those found in the other species studied.

When paper VII had already been submitted for publication, a very similar investigation on a histamine-methylating enzyme from guinea-pig brain appeared, by Brown, Tomchick and Axelrod (1959). Brown *et al.* use the name imidazole N-methyl-transferase for the histamine-methylating enzyme, although several different imidazole derivatives as well as imidazole itself were tested as substrates with negative result. Therefore the name histamine methyl-transferase will be retained by the present author, until a better alternative becomes available.

Brown *et al.* reported that histamine methyl-transferase from guinea-pig brain is inhibited 75 % or more by serotonin, chlorpromazine and brom-lysergic acid diethylamide in relatively low concentrations (about $5 \cdot 10^{-5}$ *M*). Their experiments indicate that the inhibition by serotonin is possibly non-competitive, although this has not been definitely established. It does not seem very likely that serotonin is a substrate of histamine methyl-transferase. These findings might have interesting implications

but so far nothing is known about the effect of these substances on the histamine metabolism *in vivo*.

Histamine methyl-transferase is present in varying amounts in most organs of the common laboratory animals (Brown *et al.* 1959, Lindahl, unpublished results). Enzymes which catalyse the O-methylation of adrenaline, nor-adrenaline and a number of catechols and the N-methylation of nicotinamide, guanidino-acetic acid and noradrenaline with the mediation of S-adenosylmethionine are also known to occur frequently (Axelrod 1959, Cantoni 1957). Thus it must be supposed that S-adenosylmethionine acts as a dispenser for methyl groups, the distribution being determined by the relative concentrations of the respective enzymes and substrates or possibly the accessibility of other cofactors, e.g. magnesium. It is then interesting to refer to the hypothesis by Schayer (1960), who suggests that circulatory homeostasis may depend upon a balance between histamine and the catechol amines.

Little can be said at present about the physiological significance of histamine methyl-transferase. So far it is evident that the methylation, together with the oxidative deamination by diamine oxidase, is a means of histamine elimination, as methylhistamine is essentially devoid of the pharmacological effects associated with histamine (Lee and Jones 1949), and the methylated derivatives appear in the urine when histamine is injected. If diamine oxidase is inhibited *in vivo* with aminoguanidine, the histamine methyl-transferase seems to take over the extra inactivating funtion.

The role of histamine methyl-transferase vis-à-vis the body histamine will await evaluation until adequate methods for determination of non-radioactive methylated histamine derivatives make it possible to study their occurrence, distribution and excretion.

In this connection the new hypothesis concerning the role of endogenous histamine which has been developed by Kahlson and his co-workers must be recalled (Kahlson 1960*a*). This hypothesis, which so far must be expressed in rather general terms, implies briefly that continuously formed, intracellular histamine somehow takes part in the metabolic processes involved in growth, repair and regneration. This histamine is spent within the cell and eliminated through enzymatic inactivation and excretion. Under conditions of rapid growth the histamine formation is enhanced to such a degree that the amine escapes to the extracellular spaces where it eventually exerts its pharmacological effects. The intermittently secreted histamine stimulating, for example, gastric secretion and the anterior pituitary gland is thought to have a different genesis.

This conception is based on the discovery of an immense increase of histamine formation in the foetus (c.f. also Misrahy 1946), in granulation tissue and in the regenerating liver. In the search for a conceivable role of histamine methyl-transferase it might be advantageous to connect up with these findings. It is recalled that the synthesis of S-adenosylmethionine seems to be greatly enhanced in regenerating liver (Mutolo, Maggio and Giudice 1958). In fact the turnover rate of S-adenosylmethionine may be expected to increase in all rapidly growing tissues on account of their higher metabolic activity.

3. Histamine metabolism in human pregnancy

Normal human blood or plasma cannot or only to a very small extent transform histamine into pharmacologically inactive derivatives *in vitro*. Plasma obtained from pregnant women, on the other hand, has a high histaminolytic activity. Furthermore extracts of placental tissue destroy histamine rapidly (for references see Swanberg 1950). As far as I am aware, no other physiological or pathological condition is known to induce such a drastic increase of the histaminolytic power. It was therefore natural to investigate as the first application the possible role of histamine methyl-transferase in the histamine metabolism during pregnancy (papers VIII and IX).

The histamine-destroying enzyme in placenta and pregnancy plasma, here called histaminase, is by many workers considered to be identical with diamine oxidase (see Zeller 1956). However, Kapeller-Adler has not accepted this view but claims that the degradation of histamine and diamines, e.g., cadaverine are catalysed by different enzymes (Kapeller-Adler and Renwick 1956). The experimental reasons for these opinions seem not quite conclusive (for discussion see the Ciba Foundation Symposium on Histamine, Churchill 1956, pp. 258–262 and 339–380).

The primary metabolite formed by the action of purified diamine oxidase from hog kidney on histamine is imidazoleacetaldehyde (Tabor 1951, Kapeller-Adler and Fletcher 1959). Some time ago it was suggested that imidazoleacetaldehyde is formed also by the action of histaminase in pregnancy plasma (Lindahl *et al.* 1958). Recently Kapeller-Adler (1960) reported that imidazoleacetaldehyde is the only histamine metabolite formed also by the action of placental histaminase. However, this cannot be taken as evidence that pregnancy histaminase is identical with diamine oxidase: the same metabolite may be formed through the action of at least three different enzymes which catalyse the oxidative removal of the side-chain amino group of histamine (see section 2).

The interpretation of the possible function of the histaminase in the histamine metabolism during pregnancy is somewhat controversial. On the one hand, it is claimed that the susceptibility to injected histamine is not diminished during pregnancy (for references see Kahlson 1956). This would be explained if histaminase was inactive *in vivo*. In fact experimental evidence in favour of this assumption has been presented, although concerning only the enzymic activity in the blood-stream (see Kahlson 1956 and 1960*b*). On the other hand, it has been demonstrated that the histamine formation is greatly increased during pregnancy, but somehow the mother is protected against its noxious effects (Kahlson 1960*a*). As the urinary excretion of histamine is not increased during human pregnancy (Rockenschaub 1953), these findings may speak in favour of increased histaminase activity. The problem was made no less intriguing by recent investigations which revealed that the metabolic pattern of injected histamine is mainly the same in pregnant and non-pregnant women as judged from its metabolites in the urine (Nilsson *et al.* 1959).

Some of these discrepancies would find a logical explanation, if, in addition to histaminase, there existed an increased capacity for histamine methylation during pregnancy. This problem was studied in papers VIII and IX.

The experiments reported in paper VIII verify that there is a rapid oxidation of

histamine in different kinds of placental extracts. Independently of Kapeller-Adler (1960), it was found that imidazoleacetaldehyde is the most likely primary metabolite. The enzyme activity was completely inhibited by aminoguanidine and to some extent by iproniazid. Tyramine, cadaverine and methylhistamine were also oxidized by placental extract. Cadaverine and methylhistamine (but not tyramine) interfered markedly with the histamine oxidation. Evidently this does not necessarily prove that these amines are oxidized by the same enzyme.

When the oxidation of histamine was inhibited with aminoguanidine and/or iproniazid the presence of a histamine-methylating enzyme could be demonstrated in different types of placental extracts. Methylhistamine was formed and could be identified by the isotope-dilution method of Schayer and by chromatographic methods. The oxidation of the metabolite to methylimidazoleacetic acid non-enzymatically or by diamine oxidase from pig kidney supplied further evidence of the identity.

Methylhistamine is a substrate also of the placental histaminase: the oxidation leads to another metabolite which can be oxidized non-enzymatically to methylimidazoleacetic acid. It is suggested that this metabolite is methylimidazoleacetaldehyde.

The histamine-methylating enzyme found in placenta has been shown to possess some of the properties of the histamine methyl-transferase in liver. S-adenosylmethionine is required for the process and functions with all probability as the methyl donor. The enzyme is completely inhibited by p-chloromercuribenzoate, the inhibition being abolished by glutathione. The placental enzyme, like histamine methyl-transferase from liver, seems to be part of the soluble cytoplasm. The placental enzyme is effectively extracted with slightly acid sodium acetate buffer and can be stored in the frozen state for a long time. This extract also contains oxidizing enzyme activity, which is less stable than the methylating enzyme.

The experiments in paper VIII thus demonstrate that conventional placental extracts transform histamine to imidazoleacetaldehyde. If S-adenosylmethionine is available, the histamine may also be transformed to methylhistamine and possibly methylimidazoleacetaldehyde.

It has been claimed that placental extract is devoid of aldehyde dehydrogenase (Bruns and Stüttgen 1951). However, the experiments in paper IX showed that if a hydrogen acceptor (TPN or DPN) is added, the imidazoleacetaldehydes are oxidized further to the corresponding acids. In contrast crude diamine-oxidase preparations from pig kidney are able to form imidazoleacetic acid without addition of cofactors. Possibly the last step in the histamine oxidation may be catalysed by different enzymes in the organs mentioned. In this connection it will be recalled that Zeller has suggested that the controversial reports concerning the oxidation of histamine and diamines might depend on differences in the secondary processes (Zeller 1956).

Paper IX also contains experiments which indicate that S-adenosylmethionine is actually accessible to the histamine metabolism in the placenta *in vivo*. Slices of placental tissue were incubated with histamine in a balanced salt solution without further additions. Under these conditions imidazoleacetic acid and methylimidazoleacetic acid were found to be the main metabolites. Thus addition of S-adenosylmethionine was unnecessary for the formation of the methylated derivative. It was concluded that the methyl donor is available in placental tissue.

It seems likely that the conditions in these experiments agree more closely with *in vivo* conditions than those in the experiments involving homogenization of the tissue (paper VIII, Kapeller-Adler 1960). It is therefore suggested that the histamine

metabolites in the functioning placenta are imidazoleacetic acid and methylimidazoleacetic acid.

In conclusion the following suggestions might be advanced: the histaminase in pregnancy plasma, which is leaking out of the placenta is inefficient in the blood. Therefore it does not cause a decreased susceptibility to injected histamine. Histamine which enters the placenta is inactivated partly by oxidative deamination and partly by methylation prior to oxidation, just as in the human body in general. Thus the ratio between the different metabolites of histamine remains unchanged during pregnancy in spite of the increased total capacity for histamine inactivation.

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